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**Notes Questions for the Unit 12, Part 3 Notes: Biotechnology B**

Ms. Ottolini, AP Biology

1. List the correct order for the following steps used in the creation of recombinant plasmids and bacterial transformation.

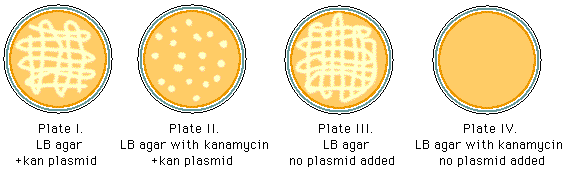
I. Transform bacteria with a recombinant DNA molecule.

II. Cut the plasmid DNA using restriction enzymes.

III. Extract plasmid DNA from bacterial cells.

IV. Hydrogen-bond the plasmid DNA to nonplasmid DNA fragments.

V. Use ligase to seal plasmid DNA to nonplasmid DNA.

In a molecular biology laboratory, a student obtained *E. coli* bacteria cells and used a common transformation procedure to induce the uptake of plasmid DNA with a gene for resistance to the antibiotic kanamycin. The results below were obtained.  


1. What results do we see on Plate I and why?
2. What results do we see on Plate II and why?
3. What results do we see on Plate III and why?
4. What results do we see on Plate IV and why?

**After the AP Test, we will be completing a bacterial transformation lab called the “PGLO” lab (created by BioRad). Below, I have given you some background information about the lab. You will be asked to make predictions regarding the lab results after reading the background information.**

**Lab Overall Description:**

***Introduction to Transformation -*** Genetic transformation is taking genes from one organism and putting them

in another. A gene is a piece of DNA that gives the instructions for making a protein. This protein gives an organism a

certain trait. A gene is inserted into an organism in order to change the organism’s trait. Genetic transformation is used

in many areas of biotechnology. In agriculture, genes coding for traits such as frost, pest, or spoilage resistance can be

inserted into plants. In medicine, gene therapy treats diseases caused by defective genes by inserting healthy copies of

the defective gene in a sick person’s cells.

You will transform bacteria with a gene that codes for Green Fluorescent Protein (GFP). The real-life source of this

gene is a jellyfish. GFP causes certain areas of the jellyfish to glow in the dark. After you transform the bacteria, they

will express their new jellyfish gene and produce the fluorescent protein. It causes them to glow a brilliant green color

under ultraviolet light.

You will learn about the process of moving genes from one organism to another with the aid of a plasmid, a small

circular piece of DNA. Bacteria have one large chromosome and one or more plasmids. Plasmids usually contain genes

for traits that may help the bacteria survive. In nature, bacteria can transfer plasmids back and forth allowing them to

share these beneficial genes. This process allows bacteria to adapt to new environments.

Bio-Rad’s pGLO plasmid has three special genes: one for GFP, a gene for antibiotic resistance, and a gene regulation

system. This system can be used to control when the bacteria produce fluorescent protein. The gene for GFP can be

switched on in transformed cells by adding the sugar arabinose to the cells’ food source. Transformed cells will appear

white on plates not containing arabinose, and fluorescent green when arabinose is included in the nutrient agar

medium. Also, we can test that cells have been transformed with pGLO DNA by growing them on antibiotic plates.

***The Genes -*** Genetic transformation involves the insertion of some new DNA into the E. coli cells. Bacteria

have one large chromosome and one or more plasmids. Plasmids usually contain genes for traits that may help the

bacteria survive. Scientists can use a process called genetic engineering to insert genes coding for new traits into a

plasmid. In this lab, the pGLO plasmid has the GFP gene that codes for the green fluorescent protein and a gene that

codes for a protein that gives the bacteria resistance to an antibiotic. The pGlo plasmid can then be used to transform

bacteria to give them this new trait.

***The Act of Transformation -*** This transformation procedure involves three main steps. These steps are

intended to insert the plasmid DNA into the E. coli cells and provide an environment for the cells to produce their

newly acquired genes.

A. To move the pGLO plasmid DNA through the cell membrane you will:

1.) Use a transformation solution of CaCl2 (calcium chloride) to make cells competent

Competent Cells - Bacterial cells must be in a particular state before they can be transformed. This state is

referred to as *competency*. This state can be achieved naturally in some species of bacteria when levels of nutrients and

oxygen are low. E.coli, the organism on which most current research is performed, must be artificially induced to

make it competent. Competent E. coli cells are very fragile and must be treated carefully.

Transformation Solution: Calcium Chloride CaCl2 - The bacterial cell membrane is permeable to chloride

ions, but is non-permeable to calcium ions. As the chloride ions enter the cell, water molecules accompany the charged

particle. This influx of water causes the cells to swell and is necessary for the uptake of DNA. The exact mechanism of

this uptake is unknown. It is known, however, that the calcium chloride treatment should be followed by heat.

2.) Carry out a procedure referred to as heat shock so bacteria can take in the plasmid

Heat Shock Treatment - When E.coli are subjected to 42°C heat, a set of genes are expressed which aid the

bacteria in surviving at that temperature. This set of genes is called the heat shock genes. The heat shock step is

necessary for the uptake of DNA. At temperatures above 42°C, the bacteria's ability to uptake DNA is lowered, and at

extreme temperatures the bacteria will die.

B. For transformed cells to grow in the presence of ampicillin you must:

3.) Provide nutrients and a short incubation period to begin expressing the newly acquired genes

Incubation - After the heat shock step, intact plasmid DNA molecules replicate in bacterial host cells. To help

the bacterial cells recover from the heat shock, the cells are briefly incubated in LB Nutrient Broth, a solution that

provides nutrients for the bacteria. As the cells recover, plasmid genes are expressed, including those that allow the

replication of plasmids which will end up in new, dividing bacterial cells.



***Genetic Regulation -*** Our bodies contain thousands of different proteins which perform many different jobs.

Digestive enzymes are proteins; some of the hormone signals that run through our bodies and the antibodies protecting

us from disease are proteins. The information for assembling a protein is carried in our DNA. The section of DNA

which contains the code for making a protein is called a gene. There are over 30,000–100,000 genes in the human

genome. Each gene codes for a unique protein: one gene, one protein. The gene that codes for a digestive enzyme in

your mouth is different from one that codes for an antibody or the pigment that colors your eyes.

Organisms regulate expression of their genes and ultimately the amounts and kinds of proteins present within

their cells for many reasons, including developmental changes, cellular specialization, and adaptation to the

environment. Gene regulation not only allows for adaptation to differing conditions, but also prevents wasteful

overproduction of unneeded proteins which would put the organism at a competitive disadvantage. The genes involved

in the transport and breakdown (catabolism) of food are good examples of highly regulated genes. For example, the

sugar arabinose is both a source of energy and a source of carbon. *E. coli* bacteria produce three enzymes (proteins)

needed to digest arabinose as a food source. The genes which code for these enzymes are not expressed when

arabinose is absent, but they are expressed when arabinose is present in their environment. How is this so?

Regulation of the expression of proteins often occurs at the level of transcription from DNA into RNA. This

regulation takes place at a very specific location on the DNA template, called a promoter, where RNA polymerase sits

down on the DNA and begins transcription of the gene. In bacteria, groups of related genes are often clustered

together and transcribed into RNA from one promoter. These clusters of genes controlled by a single promoter are

called operons. The three genes *(araB, araA* and *araD)* that code for three digestive enzymes involved in the

breakdown of arabinose are clustered together in what is known as the arabinose operon. When arabinose is present in

the environment, bacteria take it up. Once inside, the arabinose acts as an inducer on the arabinose operon and the

interaction causes the transcription of the three digestive enzyme genes. When the three enzymes are produced, they

break down arabinose, and eventually the arabinose runs out. In the absence of arabinose the transcription is shut off.

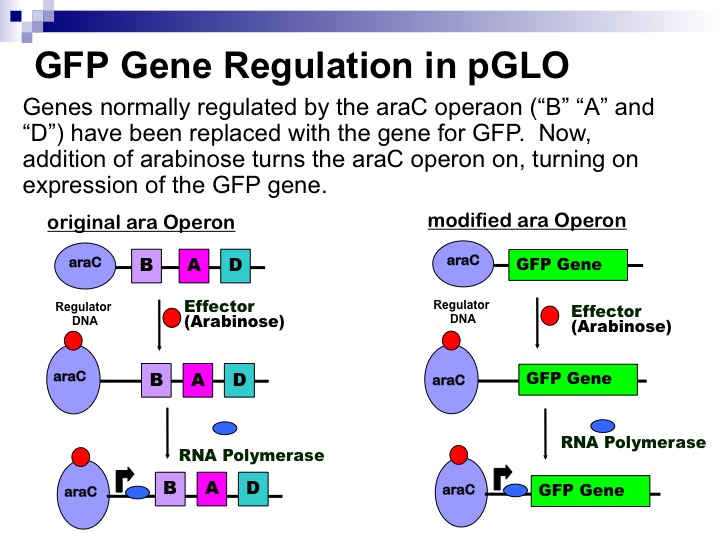
The DNA code of the pGLO plasmid has been engineered to incorporate aspects of the arabinose operon. The

genes which code for break down of arabinose, *araB, A* and *D,* have been replaced by the single gene which codes for

GFP. Therefore, in the presence of arabinose, GFP is produced. Cells fluoresce brilliant green as they produce more

and more GFP. In the absence of arabinose, GFP gene is not transcribed. When GFP is not made, bacteria colonies will

appear to have a wild-type (natural) phenotype—of white colonies with no fluorescence.



***Genetic Selection -*** Not all cells will have the plasmid and not all newly produced plasmids will end up in new

bacteria cells. So, it is necessary to select for bacterial cells which contain the plasmid. This is commonly performed

with antibiotic selection. Some E.coli strains cannot grow in the presence of common antibiotics like ampicillin.

Plasmids used for the cloning and manipulation of DNA have been engineered to contain the genes for antibiotic

resistance. Thus, if the bacterial transformation is plated onto media containing ampicillin, only bacteria which have the

plasmid DNA will have the ability to metabolize ampicillin and form colonies. In this way, bacterial cells containing

plasmid DNA are selected.

**Basic Procedure –** In the lab, you will use the following basic procedure.

1. You will create four petri dishes with the bottoms coated in LB agar. Agar is a gel-like substance that bacteria can grow on and LB is bacteria food.
2. In three of the petri dishes you will include the antibiotic ampicillin (amp) in the agar. One of the petri dishes will be used as a control so it will not have amp.
3. One of the three petri dishes with amp will also have the sugar arabinose (ara) in the agar.
4. Two of the petri dishes (one with LB/amp and the other with LB/amp/ara) will receive bacteria that HAVE been transformed with the PGLO plasmid (+PGLO). Two of the petri dishes (one with LB/amp and the other with just LB) will receive bacteria that have NOT been transformed with the PGLO plasmid (-PGLO).
5. Therefore, your petri dishes will be labeled as follows…

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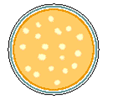
**Predicted Results –** Use the following chart to provide predictions regarding what you will see on each of the petri dishes after the bacteria have been given approximately 2 days to grow in the petri dishes.

Note: Plates with bacterial growth may show extensive growth of bacteria (i.e. growth wherever the inoculation loop spread the bacteria.) This extensive growth is called a bacterial lawn. Plates may also show “dots,” which are colonies of bacteria. The bacteria in these colonies have been successfully “transformed” and are able to live in an environment with ampicillin. Not all bacteria have been successfully transformed, and untransformed bacteria have died, explaining the blank spaces surrounding the colonies on the plate.

Below is an image of a bacterial **lawn** growing at the bottom of a petri dish…



Below is an image of bacterial **colonies** growing at the bottom of a petri dish…



+PGLO = we will be putting bacteria on these plates that have been transformed with the PGLO plasmid

-PGLO = we will be putting bacteria on these plates that have not be transformed with the PGLO plasmid

LB = nutrient broth to feed the bacteria (found on all the plates)

AMP = ampicillin, an antibiotic (should kill off any bacteria that have not been transformed with the PGLO plasmid)

Ara = arabinose, a sugar (should cause any bacteria that have been transformed with the PGLO plasmid to glow green under UV light)

Note: For the picture column, draw a circle to represent the bottom of the petri dish. Where bacterial growth occurs, make sure you clearly show whether a bacterial lawn or colonies are growing. Also, make sure to label the color –white vs. fluorescent green—of the lawn or colonies.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Petri Dish** | **Presence of Bacteria** | **Lawn or Colonies?** | **Glow?** | **Picture** | **Explanation** |
| +PGLO  LB / AMP |  |  |  |  |  |
| +PGLO  LB / AMP / Ara |  |  |  |  |  |
| -PGLO  LB/Amp |  |  |  |  |  |
| -PGLO  LB |  |  |  |  |  |

1. On which plate do you expect to find bacteria most like the original E.coli colonies (non-transformed bacteria)?
2. On which plate or plates would you expect to find genetically transformed bacteria?
3. Which plates should be compared to determine if any genetic transformation has occurred?
4. Which plate or plates would be considered control plates in this lab?

*\*\*\*Note: Most of the information in this packet is taken from other sources, not written by me (Ms. Ottolini). I have tried to find the original version of these sources and cannot find them in order to cite them. Thank you to whoever created these sources!\*\*\**